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# Determinants of ventilation and pulmonary artery pressure during early acclimatization to hypoxia in humans

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## Key points

- Lung ventilation and pulmonary artery pressure rise progressively in response to 8 h of hypoxia, changes described as ‘acclimatization to hypoxia’. Acclimatization responses differ markedly between humans for unknown reasons.
- We explored whether the magnitudes of the ventilatory and vascular responses were related, and whether the degree of acclimatization could be predicted by acute measurements of ventilatory and vascular sensitivities.
- In 80 healthy human volunteers measurements of acclimatization were made before, during, and after a sustained exposure to 8 h of isocapnic hypoxia.
- No correlation was found between measures of ventilatory and pulmonary vascular acclimatization.
- The ventilatory chemoreflex sensitivities to acute hypoxia and hypercapnia all increased in proportion to their pre-acclimatization values following 8 h of hypoxia. The peripheral (rapid) chemoreflex sensitivity to  $\text{CO}_2$ , measured before sustained hypoxia against a background of hyperoxia, was a modest predictor of ventilatory acclimatization to hypoxia. This finding has relevance to predicting human acclimatization to the hypoxia of altitude.

**Abstract** Pulmonary ventilation and pulmonary arterial pressure both rise progressively during the first few hours of human acclimatization to hypoxia. These responses are highly variable between individuals, but the origin of this variability is unknown. Here, we sought to determine whether the variabilities between different measures of response to sustained hypoxia were related, which would suggest a common source of variability. Eighty volunteers individually underwent an 8-h isocapnic exposure to hypoxia (end-tidal  $P_{\text{O}_2}=55$  Torr) in a purpose-built chamber. Measurements of ventilation and pulmonary artery systolic pressure (PASP) assessed by Doppler echocardiography were made during the exposure. Before and after the exposure, measurements were made of the ventilatory sensitivities to acute isocapnic hypoxia ( $G_{\text{pO}_2}$ ) and hyperoxic hypercapnia, the latter divided into peripheral ( $G_{\text{pCO}_2}$ ) and central ( $G_{\text{cCO}_2}$ ) components. Substantial acclimatization was observed in both ventilation and PASP, the latter being 40% greater in women than men. No correlation was found between the magnitudes of pulmonary ventilatory and pulmonary vascular responses. For  $G_{\text{pO}_2}$ ,  $G_{\text{pCO}_2}$  and  $G_{\text{cCO}_2}$ , but not the sensitivity of PASP to acute hypoxia, the magnitude of the increase during acclimatization was proportional to the pre-acclimatization value. Additionally, the change in  $G_{\text{pO}_2}$  during acclimatization to hypoxia correlated well with most other measures of ventilatory acclimatization. Of the initial measurements prior to sustained hypoxia, only  $G_{\text{pCO}_2}$  predicted the subsequent rise in ventilation and change in  $G_{\text{pO}_2}$  during acclimatization. We conclude that the magnitudes of the ventilatory and pulmonary vascular responses to sustained hypoxia are predominantly determined by different factors and that the initial  $G_{\text{pCO}_2}$  is a modest predictor of ventilatory acclimatization.

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**Abbreviations** AM, before an 8-h period of acclimatization to hypoxia; CI, confidence interval; HIF, hypoxia-inducible factor; PASP, pulmonary artery systolic pressure; PM, after an 8-h period of acclimatization to hypoxia; VAH, ventilatory acclimatization to hypoxia.

## Introduction

Exposure to hypoxia for periods longer than ~1 h generates progressive changes in a number of physiological systems. These include changes in respiratory control, which result in the phenomenon of ventilatory acclimatization to hypoxia (Douglas *et al.* 1913; Howard & Robbins, 1995b; Powell *et al.* 1998) and changes in the pulmonary vasculature, which result in the development of pulmonary hypertension (Kronenberg *et al.* 1971; Dorrington *et al.* 1997; Dehnert *et al.* 2005). It is well recognized that humans vary markedly in the magnitudes of these responses to sustained hypoxia, but the origins of this variation remain unclear. Related to this but from a clinical perspective, Bärtsch and Swenson concluded in a recent review that 'there are currently no reliable tests to predict susceptibility to high-altitude illnesses during ascent' (Bärtsch & Swenson, 2013).

There are a number of possible approaches, each with its own advantages and limitations, to try to gain insight into the origins of the marked variability in human responses to hypoxia. The approach we have adopted in this study has been to undertake detailed physiological measurements in a large number of volunteers in relation to the ventilatory and pulmonary vascular control systems, and examine how these measurements change before *versus* after a highly standardized exposure to sustained isocapnic hypoxia. The purpose is then to look for relationships between these different measurements. If such relationships are present, then this suggests not only that one or more physiological mechanisms are common to the measurements, but also that the variations within those mechanisms between individuals are sufficiently large to contribute to the overall variability in the responses to sustained hypoxia.

There are two broad scenarios. First, one or more measurements made before exposure to sustained hypoxia could predict the magnitude of change in one or more variables during exposure to hypoxia. Second, the magnitudes of change during sustained hypoxia in two or more variables could be related to one another.

Sources of common variation can be viewed as arising at molecular, cellular and systems levels. An example at the molecular level is that there may be significant variation between individuals in the responsiveness of the

hypoxia-inducible factor (HIF) transcription activation system (Brooks *et al.* 2009). As this molecular mechanism underlies the progressive responses to sustained hypoxia in a number of systems, it follows that significant variability in this mechanism between individuals is likely to result in correlation between the various systems-level responses to sustained hypoxia. On the other hand, absence of correlation would suggest that, whatever biochemical variation exists between individuals in the HIF system, it is insufficient to generate significant physiological correlation at the systems level. At a more integrative level, the carotid body is important to ventilatory acclimatization (Smith *et al.* 1986) and may play some role in modulating the pulmonary vascular response to sustained hypoxia (Levitzky, 1979; Albert & Swenson, 2014). Thus, if variations in carotid body function between individuals are physiologically significant, then this will induce correlation between a number of responses to sustained hypoxia.

We studied 80 volunteers, each on an individual basis so that we could both standardize the 8-h hypoxic stimulus within the alveolar gas and make detailed physiological measurements before, during and after the 8-h stimulus. We maintained isocapnia throughout the 8-h hypoxic exposures in order to enable us to observe the effects of sustained hypoxia whilst avoiding confounding influences associated with the changes in acid-base status that take place when hypocapnia is permitted to occur in response to an increase in ventilation. Measurements included the well-characterized parameters of the chemoreflex control system known to change during such exposure, including the acute ventilatory sensitivities to both hypoxia and hypercapnia, and also the basal level of ventilation present in the absence of hypoxia and hypercapnia (Howard & Robbins, 1995a; Fatemian & Robbins, 1998; Fatemian *et al.* 2001). Pulmonary vascular responses have been measured via the established non-invasive method of Doppler echocardiography (Peacock *et al.* 1990; Balanos *et al.* 2002; Croft *et al.* 2013). We found a striking absence of correlation between the measures of ventilatory acclimatization to hypoxia and changes in the pulmonary vasculature. The study revealed a novel predictor of ventilatory acclimatization: the peripheral chemoreflex sensitivity to CO<sub>2</sub> against a background of hypoxia.

## Methods

### Participants

Volunteers were required to be non-smokers between the ages of 18 and 50 years, in good general health, and with no significant cardiovascular or respiratory disease. All potential volunteers were assessed to determine whether they had tricuspid regurgitation detectable by Doppler echocardiography, as needed for the indirect assessment of pulmonary artery pressure. They then underwent preliminary measurements of their air-breathing end-tidal  $P_{\text{CO}_2}$  ( $P_{\text{ETCO}_2}$ ) and their ventilatory responses to acute hypoxia and hypercapnia. These measurements helped to familiarize the volunteers with the laboratory environment and equipment, and also gave them an early opportunity to decide not to participate in the study if they felt uncomfortable with any of the procedures. In all, 88 healthy volunteers, male and female, entered the study. The studies in women were not limited to one phase of the menstrual cycle. Of these volunteers, eight did not finish because of adverse reactions to the hypoxic exposure, such as sickness and headache. The study was conducted in accordance with the *Declaration of Helsinki* and had been approved by the Central Oxford Research Ethics Committee. Informed written consent was obtained before any of the preliminary measurements were made or the main experiment was undertaken.

### Outline of main experimental protocol

Measurements were made before (AM) and after (PM) an 8-h period of acclimatization to hypoxia on a single experimental day.

**Pre-acclimatization (AM) measurements.** These measurements were made in the following order: (1) air-breathing  $P_{\text{ETCO}_2}$ ; (2) maximum pressure difference across tricuspid valve (via echocardiography) while breathing air; (3) ventilatory sensitivity to hypoxia; and (4) ventilatory sensitivity to hypercapnia. There was an interval of at least 10 min between finishing the measurement of the ventilatory sensitivity to hypoxia and starting the measurement of the ventilatory sensitivity to hypercapnia.

**Acclimatization period.** There was an interval of at least 10 min between finishing the AM measurements and starting the process of acclimatization. The process of acclimatization lasted for 8 h, and during this period the participant's  $P_{\text{ETO}_2}$  was held at 55 Torr and  $P_{\text{ETCO}_2}$  was held at the same level as the air-breathing value measured in step 1 above. Ventilation and cardiovascular measurements were made at 20 min, 1 h, and then hourly intervals during the 8-h exposure.

**Post-acclimatization (PM) measurements.** After 8 h the participant left the chamber and was allowed to rest (breathing room air) for 30 min. Then the post-acclimatization (PM) measurements were made, in exactly the same manner as the AM measurements.

### Experimental techniques

**Measurements of air-breathing  $P_{\text{ETCO}_2}$ .** Nasal measurements of air-breathing  $P_{\text{ETCO}_2}$  were made by capnography whilst the volunteer was sitting quietly (Datex Normcap Oxy; Datex, Helsinki, Finland).

**Echocardiographic measurements of cardiovascular function.** Pulmonary artery systolic pressure (PASP) was determined using a standard Doppler technique. A Sonos 5500 (Hewlett-Packard, Boston, MA, USA) or a Vivid-i (GE Medical Systems, Chalfont St Giles, UK) echocardiography machine was used to estimate the maximum systolic pressure gradient across the tricuspid valve and PASP was calculated using the modified Bernoulli equation and an estimated right atrial pressure of 5 Torr (Smith *et al.* 2008).

**Measurements of acute ventilatory sensitivities to hypoxia and hypercapnia.** These measurements were made using the technique of dynamic end-tidal forcing (Robbins *et al.* 1982). During this procedure, the volunteer breathed through a mouth-piece whilst wearing a nose clip. The respired gases were sampled continuously and analysed by mass spectrometry. The end-tidal values were detected in real time and used to provide feedback to a computer-controlled gas mixing system. This mixing system was used to generate the inspiratory gas mixtures on a breath-by-breath basis to force the end-tidal gases to follow the appropriate pattern for each protocol. Tidal volumes were measured using a turbine volume-sensing device.

The ventilatory sensitivity to hypoxia was measured using standard methodology by exposure to a 12-min square wave variation in  $P_{\text{ETO}_2}$  alternating between 1 min of euoxia (100 Torr) and 1 min of hypoxia (50 Torr) while  $P_{\text{ETCO}_2}$  was held constant at  $\sim 2$  Torr above the volunteer's natural air-breathing  $P_{\text{ETCO}_2}$  (Ren *et al.* 2000). There was a 5-min lead-in period ( $P_{\text{ETO}_2} = 100$  Torr,  $P_{\text{ETCO}_2} = \text{air-breathing } P_{\text{ETCO}_2} + 2$  Torr) before the start of the square wave in order to allow ventilation to reach a steady level in response to the slight increase in  $P_{\text{ETCO}_2}$ .

The ventilatory sensitivity to hypercapnia was measured using the established technique of exposure to a specifically designed multi-frequency binary sequence variation in  $P_{\text{ETCO}_2}$  which alternated between 2 and 10 Torr above the volunteer's air-breathing  $P_{\text{ETCO}_2}$  (Pedersen *et al.* 1999).  $P_{\text{ETO}_2}$  was held constant during this sequence at 250 Torr.

There was a 5-min lead-in period ( $P_{\text{ETO}_2} = 250$  Torr,  $P_{\text{ETCO}_2} = \text{air-breathing } P_{\text{ETCO}_2} + 2$  Torr) before the start of the sequence to allow any ventilatory response to the raised level of oxygen to reach a steady level.

**Acclimatization to hypoxia.** The 8-h acclimatization to hypoxia was undertaken by modifying the composition of the atmosphere in a purpose-built chamber (Howard *et al.* 1995). Inside the chamber, the volunteer wore a pulse oximeter probe and nasal cannulae from which the respired gases could be continuously sampled and analysed. At the start of the experiment, the composition of gas in the chamber was set at a level likely to produce the desired  $P_{\text{ETCO}_2}$  and  $P_{\text{ETO}_2}$ . These values were also entered into the controlling computer. Then, at 5-min intervals, the controlling computer adjusted the gas composition of the chamber in a manner to minimize the deviation of the measured end-tidal values from the desired  $P_{\text{ETCO}_2}$  and  $P_{\text{ETO}_2}$ . Breath-by-breath values for inspiratory and end-tidal gas partial pressures and arterial saturation were recorded by the controlling computer.

Ventilation (exhaled)  $\dot{V}_E$  was measured 20 min after the start of the exposure, at 1 h, and then at hourly intervals thereafter. During this procedure, the volunteer wore a nose clip and breathed through a mouthpiece connected to a turbine volume-measuring device.

## Data analysis

**Modelling.** The assessments of ventilatory sensitivity to hypoxia and hypercapnia provided breath-by-breath measurements of pulmonary ventilation in response to the variations in  $P_{\text{ETO}_2}$  or  $P_{\text{ETCO}_2}$ . To obtain parameters describing peripheral and central chemoreceptor responses from these results, mathematical models of the chemoreflexes were fitted to the data. A simple first-order model (Clement & Robbins, 1993; Ren *et al.* 2000) was used for hypoxic sensitivity, with  $G_{\text{pO}_2}$  the model gain, representing the peripheral chemosensitivity to hypoxia (taken to be the total chemoreflex sensitivity). A two-compartment first-order model (Pedersen *et al.* 1999) was fitted to hypercapnia data with two gain parameters:  $G_{\text{pCO}_2}$  representing the peripheral chemosensitivity and  $G_{\text{cCO}_2}$  representing the central chemosensitivity to hypercapnia. Total chemosensitivity to hypercapnia was calculated by adding the peripheral and central gains:  $G_{\text{tCO}_2} = G_{\text{pCO}_2} + G_{\text{cCO}_2}$ .

**Statistical analysis.** All statistical analysis was conducted using the SPSS statistical package. Statistical significance was assumed at  $P < 0.05$ . The Shapiro–Wilk (SW) test of normality was applied to the distributions of seven measures of acclimatization and normality taken to be confirmed where SW delivered  $P > 0.05$ . For

**Table 1. General characteristics of the volunteers who took part in the study**

	Male ( <i>n</i> = 46)	Female ( <i>n</i> = 34)	Combined ( <i>n</i> = 80)
Age (years)	22 ± 3	22 ± 2	22 ± 3
Height (cm)	179 ± 6	166 ± 11	173 ± 11
Weight (kg)	73.9 ± 9.7	61.0 ± 7.4	68.4 ± 10.8
Body surface area (m <sup>2</sup> )	2.16 ± 0.16	1.87 ± 0.16	2.03 ± 0.21
Air-breathing $P_{\text{ETCO}_2}$ (Torr)	39.4 ± 2.2	36.8 ± 2.6	38.3 ± 2.7

Body surface area was calculated using formula by DuBois & DuBois (1916). Air-breathing end-tidal  $P_{\text{CO}_2}$  ( $P_{\text{ETCO}_2}$ ) is the control value measured before the experiment. Values are means ± SD.

variables showing a modest deviation from normality the  $P$  values associated with correlations must be regarded as approximate.

## Results

Thirty-four males and 46 females took part in the study. Their age range was 19–31 years with an average (mean ± SD) of 22 ± 3 years. Their physical characteristics are listed in Table 1.

## Examples of individual gas control and group physiological responses

**Chamber gas control.** An example record from an 8-h period of acclimatization to hypoxia is shown in Fig. 1. The 5-min averages for  $P_{\text{ETO}_2}$  and  $P_{\text{ETCO}_2}$  remain relatively constant at 55 Torr and at the volunteer's initial air-breathing  $P_{\text{ETCO}_2}$ , in this case 34 Torr, respectively. However, over the 8-h period the control system has steadily reduced the inspired (chamber)  $P_{\text{O}_2}$  and increased the inspired (chamber)  $P_{\text{CO}_2}$  in order to compensate for the effects of increasing ventilation over this period.

**Ventilation during 8 h of isocapnic hypoxia.** Figure 2 shows for all 80 volunteers the average ventilation measured in euoxia, and at 20 min, 1 h, and then at hourly intervals during the 8-h exposure to isocapnic hypoxia. It shows an acute rise in ventilation on initial exposure to hypoxia, followed by a gradual increase known as ventilatory acclimatization to hypoxia (VAH). Note that this increase is occurring despite the maintenance of constant end-tidal (and hence alveolar) gas composition. The final data point shows ventilation during air-breathing 20 min after leaving the chamber. Previous studies conducted in the same laboratory under similar conditions have shown no progressive changes in



ventilation over 8 h in air-breathing controls (Howard & Robbins, 1995b).

**Pulmonary artery systolic pressure during 8 h of isocapnic hypoxia.** Figure 2 shows for all volunteers PASP measured in euoxia, and at 20 min, 1 h, and then at hourly intervals during the 8-h period of isocapnic hypoxia. The pattern mimics to some extent that of the ventilation, with an acute rise in the first few minutes followed by a gradual increase constituting the ‘acclimatization’ of the pulmonary circulation to hypoxia. The final data point shows PASP during air-breathing 20 min after leaving the chamber. Previous studies conducted in the same laboratory under similar conditions have shown no progressive changes in PASP over 8 h in air-breathing controls (Balanos *et al.* 2002).

We use the term ‘pulmonary vascular acclimatization’ to refer to the gradual increase in pulmonary artery pressure during sustained hypoxia, even though, unlike VAH, it may be clinically unhelpful in the process of an individual becoming accustomed, or acclimatized, to prolonged hypoxia.

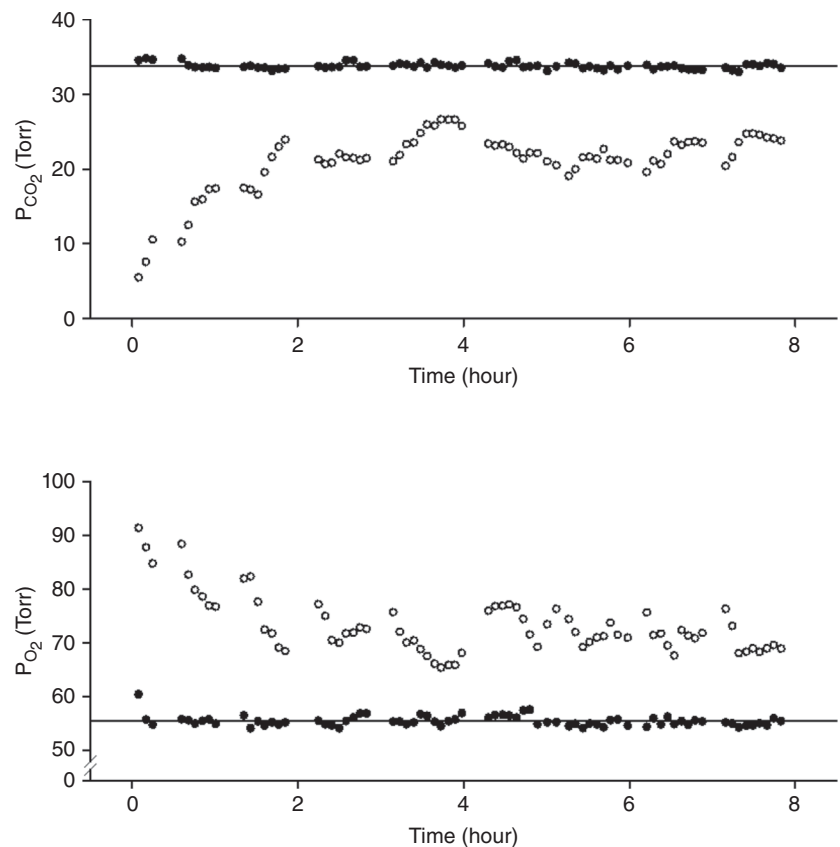
**Relationship between ventilatory and pulmonary vascular response to hypoxia at 1 h.** We examined

whether the early rise in PASP at 1 h ( $\text{PASP (1 h)} - \text{PASP (AM)}$ ) was correlated with the rise in ventilation at 1 h ( $\dot{V}_E (1 \text{ h}) - \dot{V}_E (\text{AM})$ ). No significant correlation was found ( $r = 0.16$ ,  $P = 0.19$ ).

#### Ventilatory responses to acute hypoxia and hypercapnia.

Example records from one volunteer for the ventilatory responses to acute hypoxia and hypercapnia before and after the 8-h period of acclimatization to isocapnic hypoxia are shown in Fig. 3. The records for  $P_{\text{ETO}_2}$  and  $P_{\text{ETCO}_2}$  show that the dynamic end-tidal forcing system produced well-controlled stepwise binary variation in these variables. The effects of both the steps in end-tidal gases and of acclimatization can be clearly observed in the breath-by-breath data. The lines indicate the response of the best fit model to the same stimulation in  $P_{\text{ETCO}_2}$  and  $P_{\text{ETO}_2}$  and show that it has been successful in describing the responses observed.

**Relationship between the baseline acute ventilatory sensitivity to hypoxia and the pulmonary vascular response to hypoxia at 1 h.** We examined whether the early rise in PASP at 1 h ( $\text{PASP (1 h)} - \text{PASP (AM)}$ ) was correlated with the AM measurement of the acute



**Figure 1.** Example record for one volunteer of the inspired (open circles) and end-tidal (filled circles) gas partial pressures averaged every 5 min, during the 8-h period of acclimatization to hypoxia. Note that the end-tidal values have been maintained relatively constant throughout, but the inspired – end-tidal differences progressively narrow over time as ventilation increases with acclimatization.

isocapnic hypoxic ventilatory response,  $G_{\text{pO}_2}$  (AM). No significant correlation was found ( $r = 0.20$ ,  $P = 0.08$ ).

### Measures of acclimatization

During the 8-h period of acclimatization to hypoxia, air-breathing  $P_{\text{ETCO}_2}$  fell from  $38.3 \pm 2.7$  to  $36.6 \pm 2.6$  Torr (mean  $\pm$  SD,  $P < 0.001$ ). Mean values for a range of model parameters before and after acclimatization are given in Table 2. Significant acclimatization effects were observed in many of the parameters, including ventilatory sensitivities to hypoxia and hypercapnia and the sensitivity of PASP to hypoxia.

In Fig. 4 we have summarized for all volunteers the distributions as whisker–box plots of seven different measures of acclimatization, six in relation to ventilatory control, and one in relation to PASP.

For ventilation (Fig. 4B) the variable ( $\dot{V}_E$  (8 h) –  $\dot{V}_E$  (1 h)) was adopted because the acute ventilatory response to isocapnic hypoxia, and a subsequent period of hypoxic ventilatory decline, also in the presence of isocapnic hypoxia (Easton *et al.* 1986), are completed well within the first hour of exposure. The change in ventilation over the subsequent 7 h can be regarded as a measure of VAH (Howard & Robbins, 1995b). For PASP (Fig. 4G) the variable (PASP (8 h) – PASP (1 h)) was adopted because

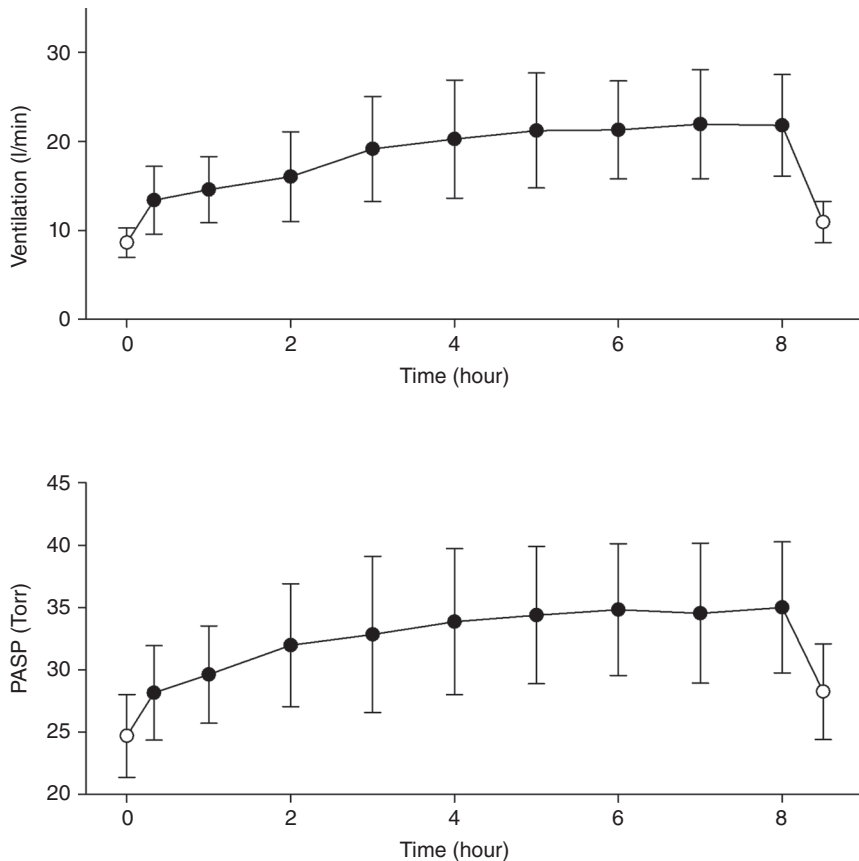
the acute pulmonary vascular response to isocapnic hypoxia is completed within approximately 10 min of exposure, and the subsequent onset of an intensification of hypoxic pulmonary vasoconstriction begins after approximately 40 min of exposure to hypoxia (Talbot *et al.* 2005). The change in PASP over the final 7 h can be regarded as a measure of pulmonary vascular acclimatization to hypoxia. The confidence interval for all seven measures of acclimatization excludes zero.

### Relationship between pulmonary vascular and ventilatory acclimatization

Table 3 shows the correlations between our measure of pulmonary vascular acclimatization and our six measures of ventilatory acclimatization. No measure of ventilatory acclimatization correlated significantly with the change of PASP between 1 and 8 h.

### Relationship between different measures of ventilatory acclimatization

Table 4 shows the correlations between the different measures of ventilatory acclimatization. Several of these correlations are insignificant. However, the most striking



**Figure 2. Ventilation (upper panel) and pulmonary artery systolic pressure (PASP) (lower panel) in euoxia (open circles) and during the 8-h period of acclimatization to hypoxia (filled circles) for all 80 volunteers**

Means  $\pm$  SD. Note that all measurements were under eucapnic conditions, at the initial air-breathing  $P_{\text{ETCO}_2}$ , except the final measurements; these were made during air-breathing 20 min after leaving the chamber, at which time volunteers tended to be relatively hypocapnic (see Results).

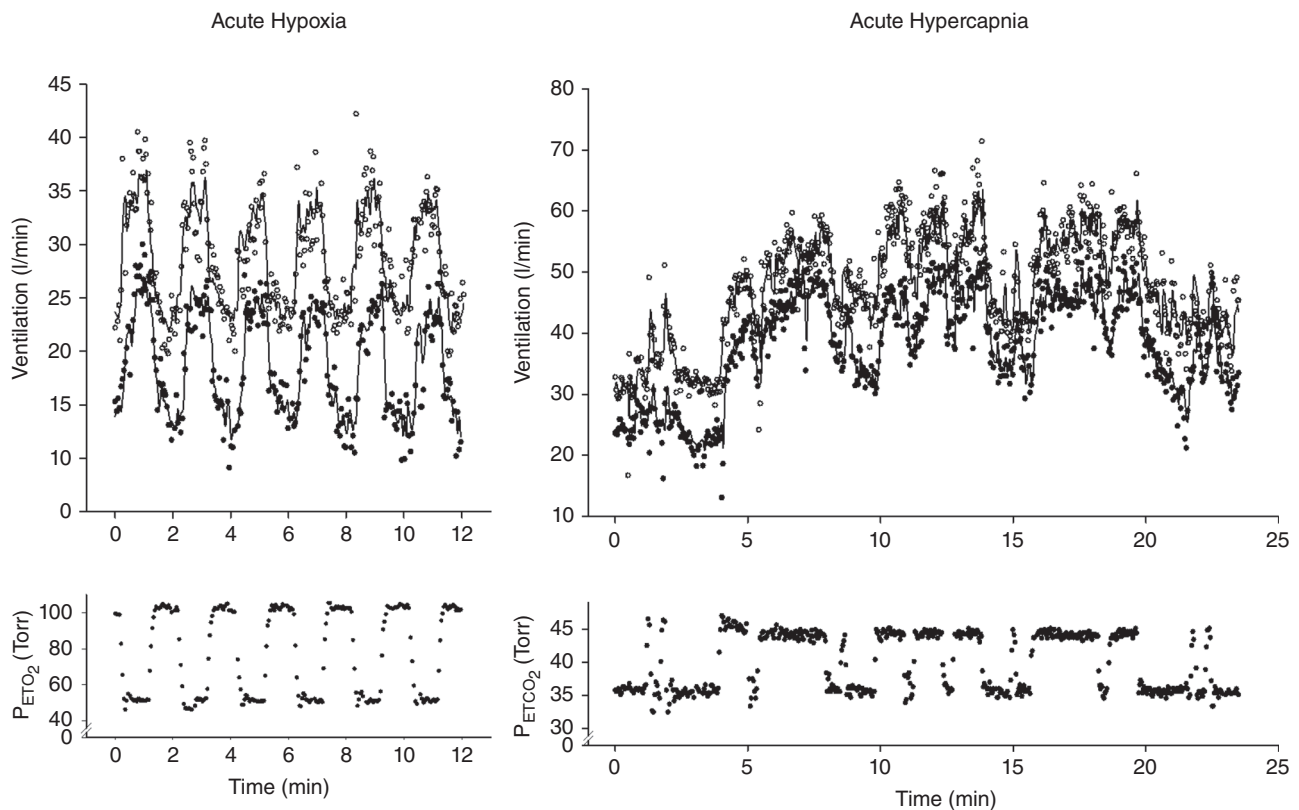
finding is that the change in acute ventilatory sensitivity to hypoxia with acclimatization ( $G_{\text{pO}_2}$ ) correlates either significantly, or almost significantly, with every other measure of VAH. This finding is consistent with the notion that changes in the acute ventilatory sensitivity to hypoxia may be driving the other ventilatory measures of acclimatization.

### Relationship between ventilatory and pulmonary vascular sensitivities to hypoxia before and after acclimatization

We separately examined the relationship between ventilatory and pulmonary vascular sensitivities to hypoxia before and after acclimatization. This analysis is shown in Fig. 5. The first four plots in this figure show the PM measures of the four parameters of chemoreflex sensitivity,  $G_{\text{pO}_2}$ ,  $G_{\text{tCO}_2}$ ,  $G_{\text{pCO}_2}$  and  $G_{\text{cCO}_2}$ , plotted against their AM values (Fig. 5A–D). Each shows a significant correlation between the PM and AM values, a slope significantly greater than unity, and an intercept that does not deviate

significantly from the origin. These results indicate that the magnitude of change in chemoreflex sensitivity with acclimatization tends to be proportional to the initial value of the parameter.

For PASP the equivalent analysis leads to a different conclusion. This analysis is shown in Fig. 5E. Here a measure of the acute sensitivity of PASP to hypoxia is taken as the difference between PASP at 8 h in the hypoxic chamber environment and PASP measured during air-breathing shortly thereafter: (PASP (8 h) – PASP (PM)). The equivalent sensitivity before acclimatization is taken as the difference between PASP at 20 min in the hypoxic chamber environment and PASP measured during air-breathing shortly before entering the chamber: (PASP (20 min) – PASP (AM)). Here the correlation is significant, with a slope not significantly different from unity, and an intercept significantly away from the origin. This result indicates that the magnitude of the change in sensitivity of PASP to acute hypoxia with acclimatization is independent of the initial sensitivity before acclimatization.



**Figure 3.** Example records from one volunteer of the assessment of acute ventilatory sensitivities to hypoxia (left panels) and hypercapnia (right panels)

Upper panels, breath by breath ventilation before (filled circles) and after (open circles) acclimatization. Data are shown as points and model fits as continuous lines. Lower panels illustrate the corresponding gas profiles (shown only for data before acclimatization); note the square wave variation in  $P_{\text{ETCO}_2}$  (left) and the multi-frequency binary sequence in  $P_{\text{ETCO}_2}$  (right).



**Table 2. Fitted parameters for chemoreflex models and measured pulmonary artery systolic pressure (PASP) before and after exposure to 8 h of hypoxia**

	AM	PM	P value
Chemoreflex responses to hypoxia			
$G_{pO_2}$ (l min <sup>-1</sup> (%) <sup>-1</sup> )	0.69 ± 0.36	1.24 ± 0.56	<0.001
$\dot{V}_E$ (l min <sup>-1</sup> )	10.14 ± 2.74	15.81 ± 4.61	<0.001
$\tau_{pO_2}$ (s)	9.2 ± 6.1	13.4 ± 5.7	<0.001
$d_{pO_2}$ (s)	5.0 ± 2.2	3.9 ± 1.4	<0.001
$F$	0.8 ± 0.2	0.8 ± 0.2	0.87
$R_v/R_w$	0.9 ± 1.0	0.9 ± 1.0	0.78
Chemoreflex responses to hypercapnia			
$G_{pCO_2}$ (l min <sup>-1</sup> Torr <sup>-1</sup> )	0.83 ± 0.37	1.09 ± 0.44	<0.001
$G_{cCO_2}$ (l min <sup>-1</sup> Torr <sup>-1</sup> )	1.41 ± 0.44	1.92 ± 0.69	<0.001
$B$ (Torr)	34.1 ± 3.6	31.4 ± 4.4	<0.001
$\tau_{pCO_2}$ (s)	19.9 ± 7.1	20.7 ± 7.0	0.48
$d_{pCO_2}$ (s)	6.1 ± 2.2	6.2 ± 2.7	0.90
$\tau_c$ (s)	232.7 ± 72.2	234.7 ± 71.3	0.85
$d_c$ (s)	11.2 ± 5.2	12.3 ± 4.9	0.12
$F$	0.9 ± 0.1	0.8 ± 0.1	0.63
$R_v/R_w$	0.4 ± 0.5	0.4 ± 0.6	0.74
Pulmonary artery systolic pressure, air breathing			
PASP (Torr)	24.6 ± 3.3	28.1 ± 3.9	<0.001
Pulmonary artery systolic pressure, hypoxia			
PASP (Torr)	28.3 ± 4.1	35.3 ± 5.1	<0.001

Hypoxia:  $G_{pO_2}$ , peripheral chemoreflex sensitivity;  $\dot{V}_E$ , ventilation in the absence of hypoxic stimulus;  $\tau_{pO_2}$ , chemoreflex time constant;  $d_{pO_2}$ , chemoreflex delay. Hypercapnia:  $G_{pCO_2}$ , peripheral chemoreflex sensitivity;  $G_{cCO_2}$ , central chemoreflex sensitivity;  $B$  extrapolated value for  $P_{ETCO_2}$  at which  $\dot{V}_E = 0$ ;  $\tau_{pCO_2}$ , peripheral chemoreflex time constant;  $d_{pCO_2}$ , peripheral chemoreflex delay;  $\tau_c$ , central chemoreflex time constant;  $d_c$ , central chemoreflex delay. For both models:  $F$ , system gain for noise component;  $R_v/R_w$ , variance ratio for process and measurement noise. AM and PM indicate measurements taken before and after 8-h acclimatization period, respectively. For PASP during hypoxia, AM and PM indicate measurements taken 20 min and 8 h after the start of acclimatization, respectively. Values are means ± SD.  $P$  value was obtained from ANOVA comparing AM and PM data.

### Predictors of the degree of acclimatization

In accord with the ventilatory findings in the previous section, we examined the correlations between all four AM measures of ventilatory sensitivity and all six PM-minus-AM measures of ventilatory acclimatization, to look for an acute measure of ventilatory sensitivity that could predict the acclimatization response. Two significant predictions were found. These are shown in Fig. 6. The acute peripheral chemoreflex sensitivity to CO<sub>2</sub> under hyperoxic conditions ( $G_{pCO_2}$  (AM)) predicts both the rise in ventilation over the acclimatization period (Fig. 6A) and the rise in peripheral chemoreflex sensitivity to hypoxia (Fig. 6B). We noted a correlation between  $G_{pCO_2}$  (AM) and  $G_{pO_2}$  (AM) with  $r = 0.47$  ( $P < 0.0001$ ). Nevertheless,

it remains the case that, despite this correlation,  $G_{pCO_2}$  (AM) but not  $G_{pO_2}$  (AM) predicted the rise in ventilation during acclimatization.

### Acclimatization response according to sex

Differences between AM and PM measurements of air-breathing  $P_{ETCO_2}$ , all ventilatory parameters and PASP were analysed for sex differences. A significant difference between males and females was revealed only for PASP (8 h) – PASP (20 min); this was taken as a measure of the complete acclimatization response following the brief early (20-min) exposure to hypoxia in the chamber ( $5.99 \pm 0.59$  Torr (mean ± SEM) for males vs.  $8.33 \pm 0.85$  Torr for females,  $P < 0.05$ ). The result suggests a difference according to sex in the pulmonary vascular acclimatization to hypoxia but not in the ventilatory acclimatization.

### Discussion

This study focused on both the within- and between-subject variability of the responses to early acclimatization to hypoxia. No relationship was found between the magnitude of early VAH and early pulmonary vascular acclimatization to hypoxia. A second result was that, during VAH, the absolute increments in the acute ventilatory sensitivities to hypoxia and hypercapnia depend upon their initial values prior to hypoxic exposure. Third, the magnitude of the peripheral chemoreflex sensitivity to hyperoxic hypercapnia ( $G_{pCO_2}$ ) predicts the magnitude of the increase in chemoreflex sensitivity to hypoxia ( $G_{pO_2}$ ) over the sustained hypoxic exposure, and, in turn, this increase in peripheral chemoreflex sensitivity to hypoxia correlates with the magnitude of most other indices of VAH.

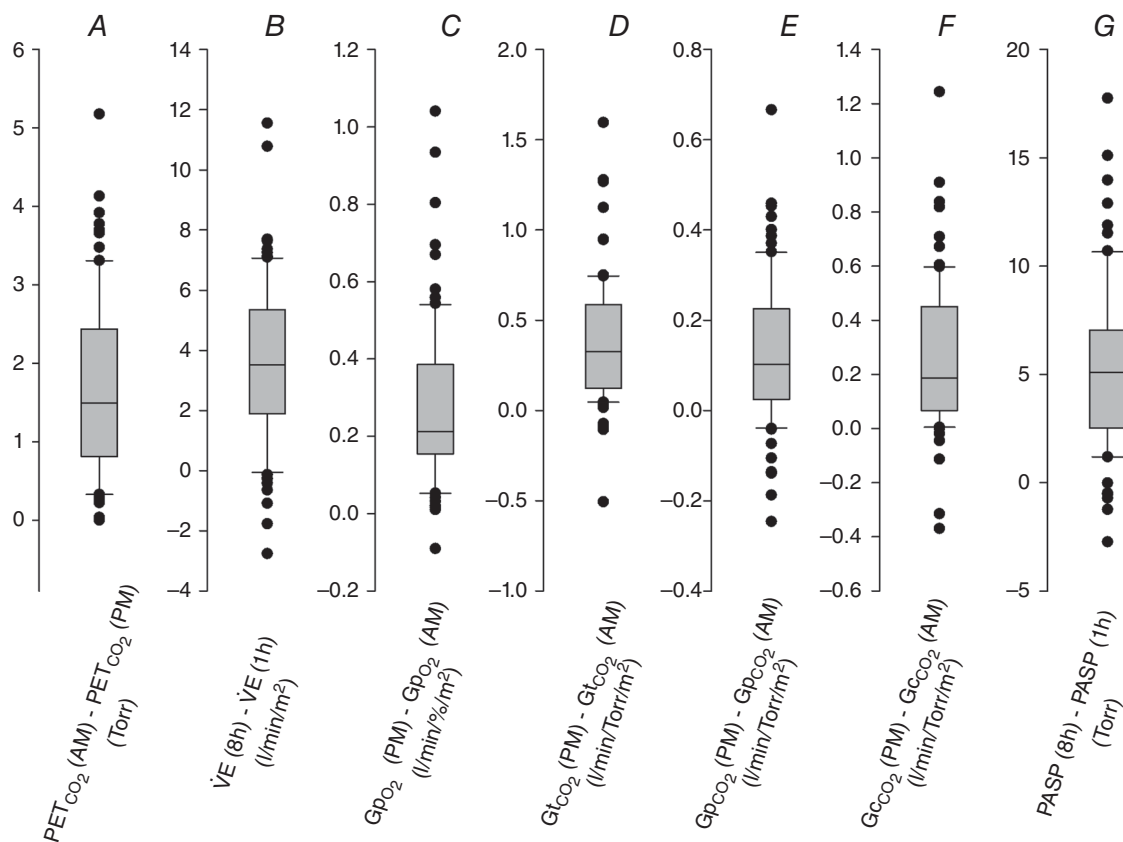
### Hypotheses for acclimatization involving respiratory alkalosis and the HIF system

For much of the 20th century, the predominant theories seeking to explain VAH involved the sensing of slow acid–base changes consequent on the initial respiratory alkalosis triggered by the exposure to hypoxia. However, a significant obstacle to these theories arose in the 1980s and 1990s, when it was shown in goats and humans, respectively, that prevention of the initial respiratory alkalosis on exposure to hypoxia did not abrogate VAH (Bisgard *et al.* 1986; Howard & Robbins, 1995b). During the 1990s, in a separate line of enquiry into the regulation of erythropoietin by hypoxia, hypoxia-inducible factor (HIF) was identified as a transcription activation factor (Semenza & Wang, 1992), and this was subsequently shown to be expressed in a wide variety of mammalian

cell lines (Maxwell *et al.* 1993). The discovery of HIF thus provided an alternative potential explanation for VAH, and indeed other slow responses to hypoxia. More direct evidence that HIF was involved in VAH came from a study of mice that were heterozygous for a functional HIF-1 allele in which these animals failed to undergo VAH when exposed to chronic hypoxia (Kline *et al.* 2002). Similarly, the pulmonary vasculature of these animals failed to respond normally to chronic hypoxia (Yu *et al.* 1999; Shimoda *et al.* 2001). In humans, the demonstration that Chuvash polycythaemia was due to germline homozygosity for a hypomorphic allele of the von Hippel–Lindau gene, and that this impaired the degradation of HIF (Ang *et al.* 2002), provided a means to examine whether increased activation of HIF under conditions of normal oxygen tension produced phenotypic changes similar to those associated with acclimatization to hypoxia. The Chuvash phenotype was found to be very similar to that of an acclimatized human,

with erythrocytosis, hyperventilation and hypocapnia, elevated pulmonary artery pressure and an increased sensitivity of both ventilation and the pulmonary vasculature to acute hypoxia (Smith *et al.* 2006).

The above results suggest that the HIF system may play a long-term role in the calibration of the ventilatory and pulmonary vascular responses to acute hypoxia. It is thus a likely candidate mechanism to generate the amplification of the ventilatory and pulmonary vascular responses to acute hypoxia following sustained hypoxic exposure observed in the current study. Thus, if there were significant biological variation in the HIF system between humans, then it might be predicted that some of the variability in acclimatization responses in the present study could relate to this, and thus potentially be common to both the ventilatory and pulmonary vascular responses. For example, genetic variants within the HIF system (PHD2, HIF2 $\alpha$ ) have already been identified as functionally highly significant



**Figure 4. Distributions (as whisker–box plots) for seven measures of acclimatization for all 80 volunteers**

The difference is taken between measurements made at the end of (8 h), or shortly after (PM), the acclimatization exposure and measurements taken before (AM), or early during (1 h) that exposure, except for A, in which the sign is reversed. A,  $P_{ETCO_2}$ , air-breathing end-tidal  $P_{CO_2}$ ; B,  $\dot{V}_E$ , ventilation; C,  $G_{PO_2}$ , peripheral chemoreceptor sensitivity to hypoxia; D–F:  $G_{TCO_2}$ ,  $G_{PCO_2}$  and  $G_{CCO_2}$ , total, peripheral and central chemoreceptor sensitivities to hypercapnia, respectively; G, PASP, pulmonary artery systolic pressure. Plots show the median, interquartile range (boxes), 95% range (bars) and individual outliers beyond the latter. Shapiro–Wilk tests of normality (suggested by  $P > 0.05$ ) yielded the following: A,  $P < 0.02$ ; B,  $P > 0.5$ ; C, D and F,  $P < 0.001$ ; E,  $P > 0.05$ ; G,  $P > 0.05$ .

Table 3. Correlations between pulmonary vascular acclimatization and different measures of ventilatory acclimatization

	$P_{\text{ETCO}_2}$ (AM) – $P_{\text{ETCO}_2}$ (PM) (Torr)	$\dot{V}_E$ (8 h) – $\dot{V}_E$ (1 h) ( $\text{l min}^{-1} \text{ m}^{-2}$ )	$G_{\text{PO}_2}$ (PM) – $G_{\text{PO}_2}$ (AM) ( $\text{l min}^{-1} (\%)^{-1} \text{ m}^{-2}$ )	$G_{\text{ICO}_2}$ (PM) – $G_{\text{ICO}_2}$ (AM) ( $\text{l min}^{-1} \text{ Torr}^{-1} \text{ m}^{-2}$ )	$G_{\text{PCO}_2}$ (PM) – $G_{\text{PCO}_2}$ (AM) ( $\text{l min}^{-1} \text{ Torr}^{-1} \text{ m}^{-2}$ )	$G_{\text{CCO}_2}$ (PM) – $G_{\text{CCO}_2}$ (AM) ( $\text{l min}^{-1} \text{ Torr}^{-1} \text{ m}^{-2}$ )
PASP (8 h) – PASP (1 h) (Torr)	$r = 0.040$ $P = 0.733$	$r = 0.035$ $P = 0.761$	$r = 0.052$ $P = 0.656$	$r = 0.025$ $P = 0.831$	$r = 0.031$ $P = 0.792$	$r = 0.048$ $P = 0.678$

PASP represents the pulmonary artery systolic pressure.  $P_{\text{ETCO}_2}$  is the air-breathing end-tidal  $P_{\text{CO}_2}$ ,  $\dot{V}_E$  is ventilation,  $G_{\text{PO}_2}$  represents peripheral chemoreceptor sensitivity to hypoxia.  $G_{\text{ICO}_2}$ ,  $G_{\text{PCO}_2}$  and  $G_{\text{CCO}_2}$  represent total, peripheral and central chemoreceptor sensitivities to hypercapnia, respectively. AM and PM indicate measurements taken before and after acclimatization period. '1 h' and '8 h' indicate measurements taken 1 h and 8 h after the start of acclimatization. Ventilations and chemoreceptor sensitivities are scaled by body surface area.  $r$  is the correlation coefficient and  $P$  is its level of significance.

for haemoglobin concentration in the Tibetan population (Beall *et al.* 2010; Simonson *et al.* 2010; Yi *et al.* 2010). The present study found no relationship between the magnitude of the changes in VAH and in the pulmonary vasculature. Thus it would appear either that the underlying sources of variation between individuals are outside of the HIF system, or else that different aspects of variation within the HIF system are important to VAH and the pulmonary vascular response to sustained hypoxia.

### The role of the carotid body in ventilatory acclimatization to hypoxia

A positive finding of this study was the discovery of a predictor for the magnitude of early VAH, namely the gain term of the fast component of the ventilatory response to hypercapnia. Through work in both experimental animals (DeGoede *et al.* 1985) and in chemodenervated humans (Fatemian *et al.* 2003), this rapid component of the ventilatory response to hypercapnia has been closely associated with the sensitivity of the carotid body chemoreflex to hypercapnia. Thus, this parameter may be seen as one measure of the strength of the carotid body chemoreflex within a particular individual, although another potential measure, the ventilatory sensitivity to acute hypoxia, did not predict the magnitude of VAH. That a parameter associated with carotid chemoreflex function predicts the magnitude of VAH fits well with previous studies demonstrating the importance of the carotid body in early VAH. These findings include demonstrations that carotid body-denervated animals do not undergo normal VAH (Forster *et al.* 1976; Lahiri *et al.* 1981), that hypoxia confined to just the carotid body is sufficient to induce early VAH (Busch *et al.* 1985) and that CNS hypoxia in the absence of carotid body hypoxia does not induce early VAH (Weizhen *et al.* 1992).

Our study found that, in general, different measures of early VAH, for example the rise in ventilation during hours 1–8 of hypoxia, the reduction in air-breathing  $P_{\text{ETCO}_2}$  after the 8-h exposure to hypoxia, and the increments in ventilatory sensitivity to  $\text{CO}_2$  ( $G_{\text{ICO}_2}$  and its components  $G_{\text{PCO}_2}$  and  $G_{\text{CCO}_2}$ ) did not correlate one with another. The clear exception to this was the increase in the acute ventilatory sensitivity to hypoxia ( $G_{\text{PO}_2}$ ), which correlated either significantly, or almost significantly ( $P < 0.06$ ), with all the above measures. This pattern of correlation suggests a causal role for the increase in the acute ventilatory sensitivity to hypoxia over the 8-h period in generating the other indices of VAH. Again, this is consistent with previous literature documenting the importance of the carotid body for early VAH cited in the preceding paragraph.

Table 4. Correlations between alternative measures of ventilatory acclimatization

	$P_{\text{ETCO}_2}(\text{AM}) - P_{\text{ETCO}_2}(\text{PM})$ (Torr)	$\dot{V}_E(8\text{ h}) - \dot{V}_E(1\text{ h})$ ( $\text{l min}^{-1} \text{ m}^{-2}$ )	$G_{\text{tCO}_2}(\text{PM}) - G_{\text{tCO}_2}(\text{AM})$ ( $\text{l min}^{-1} \text{ Torr}^{-1} \text{ m}^{-2}$ )	$G_{\text{pCO}_2}(\text{PM}) - G_{\text{pCO}_2}(\text{AM})$ ( $\text{l min}^{-1} \text{ Torr}^{-1} \text{ m}^{-2}$ )	$G_{\text{cCO}_2}(\text{PM}) - G_{\text{cCO}_2}(\text{AM})$ ( $\text{l min}^{-1} \text{ Torr}^{-1} \text{ m}^{-2}$ )
$G_{\text{pO}_2}(\text{PM}) - G_{\text{pO}_2}(\text{AM})$ ( $\text{l min}^{-1} (\%)^{-1} \text{ m}^{-2}$ )	$r = 0.258$	$r = 0.213$	$r = 0.353$	$r = 0.220$	$r = 0.320$
	$P = 0.021^*$	$P = 0.058$	$P = 0.001^*$	$P = 0.050^*$	$P = 0.004^*$
$P_{\text{ETCO}_2}(\text{AM}) - P_{\text{ETCO}_2}(\text{PM})$ (Torr)	—	$r = 0.157$	$r = 0.004$	$r = 0.097$	$r = 0.058$
	—	$P = 0.164$	$P = 0.974$	$P = 0.392$	$P = 0.608$
$\dot{V}_E(8\text{ h}) - \dot{V}_E(1\text{ h})$ ( $\text{l min}^{-1} \text{ m}^{-2}$ )	—	—	$r = 0.037$	$r = 0.161$	$r = 0.144$
	—	—	$P = 0.746$	$P = 0.154$	$P = 0.203$

Variables are as defined in Table 3.  $r$  is the correlation coefficient and  $P$  is its level of significance. \* indicates statistically significant value.

### Possible mechanisms of pulmonary vascular acclimatization to hypoxia

Hypoxic pulmonary vasoconstriction has long been regarded as an intrinsic property of the pulmonary vasculature because the response can be demonstrated in isolated vessels *in vitro* (Sylvester *et al.* 2012). However, it is unknown whether isolated pulmonary vessels would show an intensification of their vasoconstriction over several hours of hypoxia because such *in vitro* experiments have been of insufficient duration for this question to be carefully addressed.

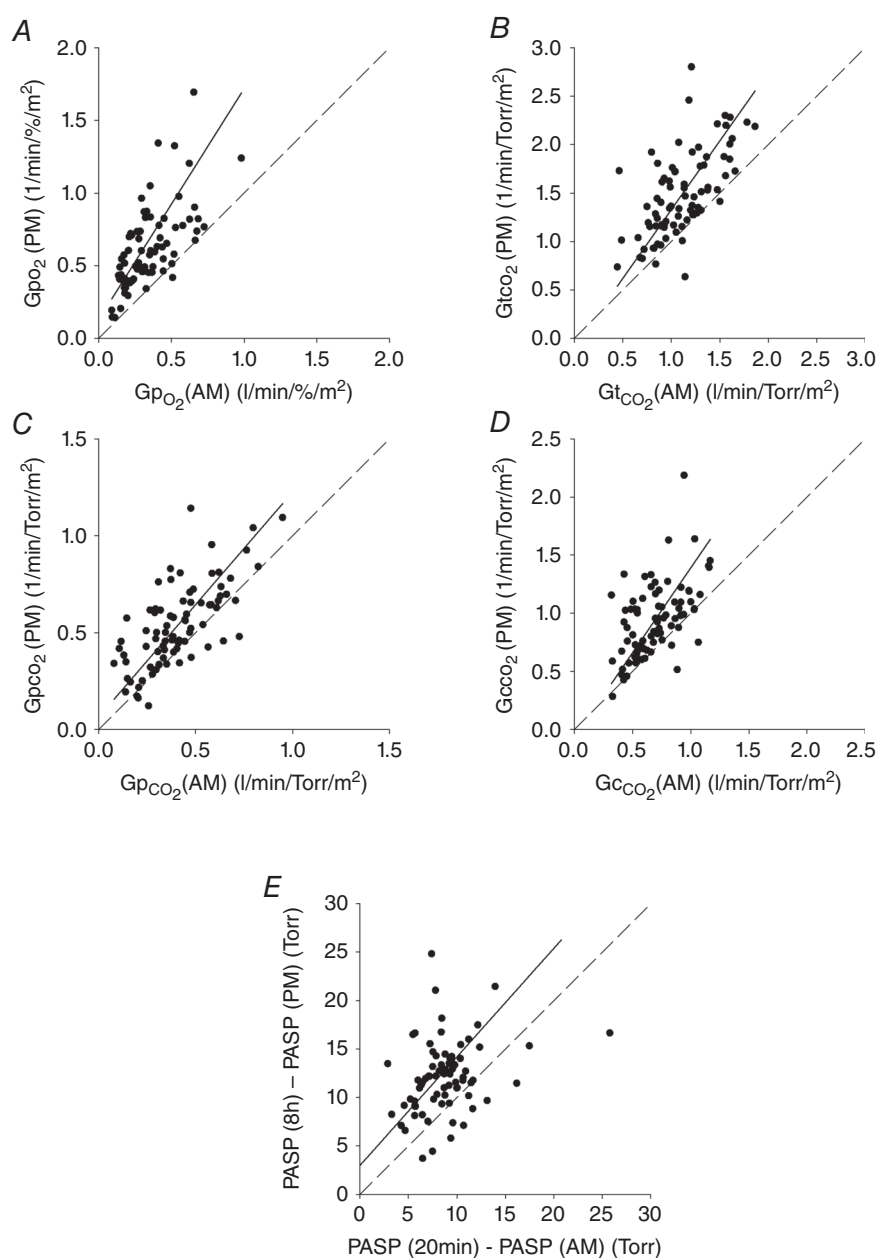
The lung vasculature is supplied with autonomic innervation that potentially makes possible the modulation of hypoxic constriction by autonomic efferents (Hebb, 1966; Downing & Lee, 1980). The work of Levitsky showed that systemic arterial hypoxaemia is associated with a chemoreflex-mediated dilatation of the pulmonary vasculature, for which there is evidence of both a parasympathetic and a sympathetic origin (Levitsky *et al.* 1978; Chapleau *et al.* 1988; Wilson & Levitsky, 1989; Brimiouille *et al.* 1997). Other studies, also in dogs, have found the opposite response (Daly & Daly, 1959), or attributed changes in hypoxic pulmonary vasoconstriction in systemic hypoxaemia primarily to changes in lung blood flow or mixed venous  $P_{\text{O}_2}$  rather than autonomic supply (Lodato *et al.* 1988; Pellett *et al.* 1997). Furthermore, it has been suggested that the innervation of the lung is not limited to the classical sympathetic and parasympathetic pathways and that sensory innervation is capable of generating peripheral terminal release of a wide range of transmitter substances that may influence vascular tone (Kummer, 2011). This may be a reason for some of the differences observed between studies that use pharmacological modulation of the sympathetic and parasympathetic mechanisms and those that interrupt afferent and efferent signalling using ablation or nerve sectioning (Naeije *et al.* 1989; Swenson, 2013; Maggiorini *et al.* 2014).

One important factor that may determine the involvement of the autonomic nervous system in the pulmonary vascular response to hypoxia is the degree and duration of hypoxia. In the present study this was exactly matched to that in a previous study of the role of the autonomic nervous system in human pulmonary vascular acclimatization (Liu *et al.* 2007). In that study autonomic blockade using the agent trimetaphan failed to abrogate the increase in basal tone and sensitivity to hypoxia induced by acclimatization with 8 h of hypoxia, suggesting that altered autonomic activity does not underlie the acclimatization observed in pulmonary vascular tone (or indeed ventilation).

Changes in cardiac output with acute and prolonged hypoxia would be expected to change PASP to some

degree. Were the pulmonary vascular resistance to remain constant, and pulmonary venous outflow pressure to be low, then the increase in PASP would be proportionate to cardiac output. However, the pulmonary circulation dilates with increases in flow, and so this simple relationship does not apply (Groves *et al.* 1987; Naeije, 2004). Two previous studies have examined this in detail during a similar level of hypoxia ( $P_{\text{ETO}_2} = 50$  Torr) and identical conditions to those used here. In a catheter-based study, pulmonary artery wedge pressure did not change during 8 h of hypoxia and PASP changed little after 2 h of hypoxia in the face of a gradually increasing cardiac output (Dorrington *et al.* 1997). In a Doppler

echocardiography-based study we examined the extent to which the rise in cardiac output during 8 h of hypoxia contributed to the rise in PASP (Balanos *et al.* 2005). In that study, the prolonged hypoxia was associated with a rise in cardiac output of  $\sim 1 \text{ l min}^{-1}$ ; the change in PASP associated with this rise in cardiac output alone, in both euoxia and hypoxia, was  $0.6 \text{ Torr l}^{-1} \text{ min}^{-1}$  (Balanos *et al.* 2005). A quantitative assessment of the size of this effect in both prolonged and acute hypoxic exposures suggests that only 5–15% of the total change in PASP may be attributed to the rise in cardiac output that is concurrent with the period of hypoxia (Balanos *et al.* 2005; Croft *et al.* 2013).



**Figure 5. Relationships between values before and after acclimatization for the acute ventilatory sensitivities to hypoxia and hypercapnia and for the sensitivity of the pulmonary arterial systolic pressure (PASP) to hypoxia**

Dots represent data from each of the 80 volunteers, the continuous lines represent the reduced major axis regression, and the dashed lines are lines of identity. A:  $G_{\text{pO}_2}$ , peripheral chemoreceptor sensitivity to hypoxia,  $r = 0.66$  ( $P < 0.001$ ), slope  $m = 1.6$  (confidence interval (CI) 1.3–1.9), intercept  $C = 0.07$  (CI  $-0.03$  to  $0.17$ ); B:  $G_{\text{tCO}_2}$ , total chemoreceptor sensitivity to hypercapnia,  $r = 0.63$  ( $P < 0.001$ ),  $m = 1.4$  (CI 1.1–1.7),  $C = -0.1$  (CI  $-0.4$  to  $0.2$ ); C:  $G_{\text{pCO}_2}$ , peripheral chemoreceptor sensitivity to hypercapnia,  $r = 0.69$  ( $P < 0.001$ ),  $m = 1.2$  (CI 1.0–1.4),  $C = 0.06$  (CI  $-0.02$  to  $0.14$ ); D:  $G_{\text{cCO}_2}$ , central chemoreceptor sensitivity to hypercapnia,  $r = 0.54$  ( $P < 0.001$ ),  $m = 1.5$  (CI 1.2–1.7),  $C = -0.1$  (CI  $-0.3$  to  $0.1$ ); E: pulmonary vascular sensitivity before and after acclimatization,  $r = 0.27$  ( $P < 0.02$ ),  $m = 1.1$  (CI 0.9–1.3),  $C = 3.0$  (CI 1.7–4.3).

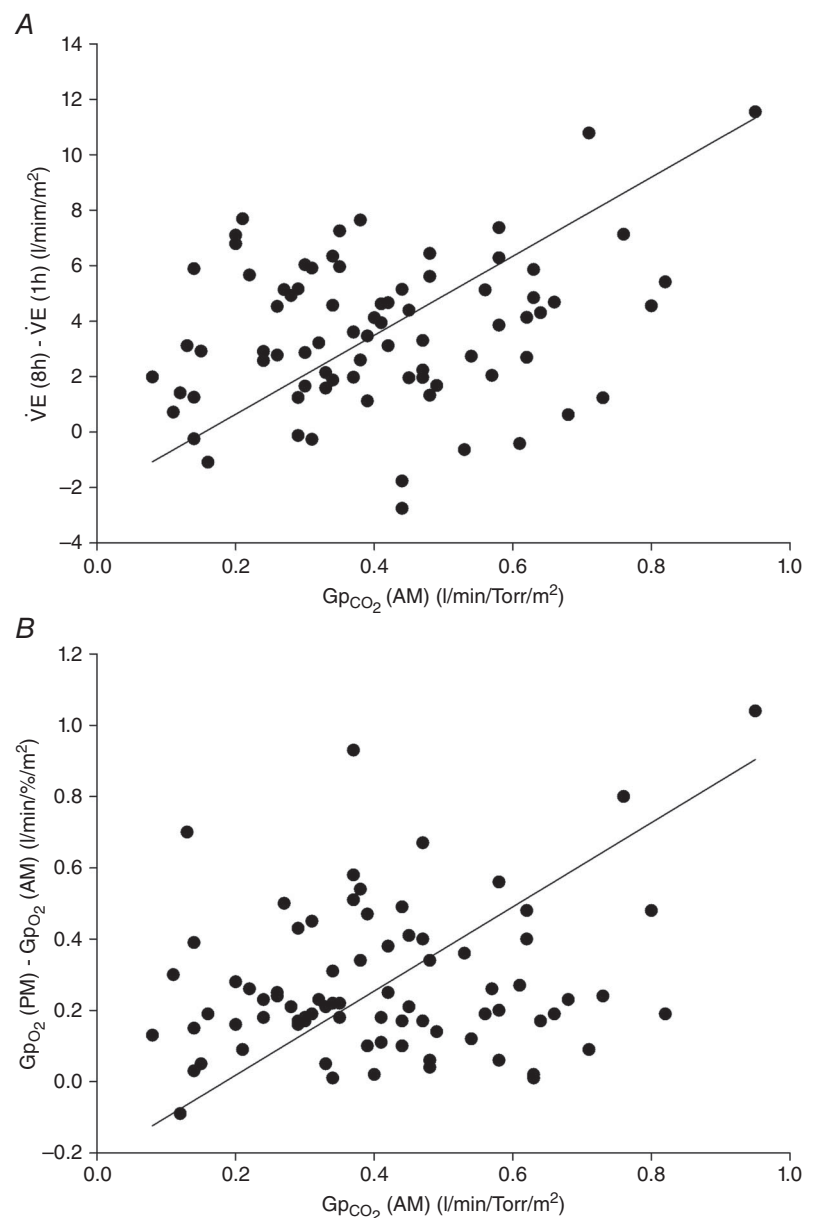


## Differences between the ventilatory and pulmonary vascular acclimatization to hypoxia

For each of the parameters describing a ventilatory sensitivity to hypoxia or hypercapnia (after normalization to body surface area), a comparison between the values after, *versus* before, the 8-h hypoxic exposure revealed that effects of the exposure on the parameter were proportionate to the pre-exposure parameter value. Therefore, for the measures of VAH based on increments in ventilatory sensitivities to hypoxia or hypercapnia a further source of variation relates to the pre-exposure value for the parameter, with larger parameter values

predisposing towards larger absolute increases with acclimatization. No such correlation was observed for systolic pulmonary arterial pressures after, *versus* before, the 8-h hypoxic exposure; the increment in pressure was independent of the starting value.

A feature of our comparison of the ventilatory and the pulmonary vascular acclimatization to hypoxia is the finding that the two physiological processes show no correlation. Perhaps in keeping with this a recent study at high altitude found no correlation between the eucapnic hypoxic ventilatory response measured at sea level and the elevation in PASP measured at an altitude of 5050 m (Hoiland *et al.* 2014).



**Figure 6. Predictions of ventilatory acclimatization from acute pre-acclimatization ventilatory sensitivity to hypercapnia ( $G_{pCO_2}(AM)$ )**

A: the increase in ventilation during 8 h of isocapnic hypoxia ( $\dot{V}_E(8h) - \dot{V}_E(1h)$ );  $r = 0.26$ ,  $P = 0.02$ ,  $m = 14.3$  (CI 11.1–17.4),  $C = -2.2$  (CI -3.6 to -0.8). B: the increase in the acute ventilatory sensitivity to hypoxia during 8 h of isocapnic hypoxia ( $G_{pO_2}(PM) - G_{pO_2}(AM)$ );  $r = 0.24$ ,  $P = 0.04$ ,  $m = 1.2$  (CI 0.9–1.4),  $C = -0.2$  (CI -0.3 to -0.1). For each graph  $r$  is the correlation coefficient and  $P$  is its level of significance,  $m$  is the slope,  $C$  is the intercept, CI refers to confidence interval. Lines show the reduced major axis regression.

This contrasts with some evidence that acute ventilatory and pulmonary vascular responses to hypoxia in humans may show an inverse relationship (Albert & Swenson, 2014), and the possibility that susceptibility to high altitude pulmonary oedema (with its associated pulmonary hypertension) may be associated with a diminished ventilatory response to hypoxia (Hohenhaus *et al.* 1995). These findings raise the possibility that experiments conducted with isocapnic hypoxia might yield different outcomes from exposures to hypoxia in which the  $P_{\text{ETCO}_2}$  is permitted to decrease in response to the elevation in ventilation, namely poikilocapnic exposures.

### Applicability of results from eucapnic experiments to poikilocapnic exposures to hypoxia

The experiments reported here involve exposures to constant alveolar hypoxia at a constant alveolar carbon dioxide. During exposure to a constant low level of inspired oxygen, as experienced for example at a fixed level of high altitude in stable atmospheric conditions, both the degree of alveolar hypoxia and the degree of alveolar hypocapnia vary as VAH develops. It is therefore of practical clinical interest to consider whether the findings obtained in the present study may also apply 'on the mountain'.

Previous experiments from our laboratory have explored changes in  $G_{\text{pO}_2}$  and  $G_{\text{pCO}_2}$  in both poikilocapnia and eucapnia in settings identical to those used in the experiments reported here. Howard & Robbins (1995a) found no significant difference between poikilocapnia and eucapnia in the change in  $G_{\text{pO}_2}$  arising during 8 h of hypoxia at the same  $P_{\text{ETO}_2}$  used in the present study (55 Torr). A later study (Fatemian & Robbins, 2001) found no significant difference between poikilocapnia and eucapnia in the change in  $G_{\text{pCO}_2}$  arising during 8 h of hypoxia at the same  $P_{\text{ETO}_2}$ . These suggest that the findings from the present study relating to these acute ventilatory sensitivities would be similar in poikilocapnic exposures. Whether the same would apply to the acute pulmonary vascular sensitivity to hypoxia remains untested.

### Acclimatization to hypoxia of the central chemoreflex response to hypercapnia

Other novel findings of this study relating to VAH include the demonstration that the slow component of the chemoreflex response to hypercapnia increased following 8 h of hypoxia. Such a possibility was suggested following a similar study on a much smaller number of subjects (Fatemian & Robbins, 2001), but that earlier study did not have the statistical power to demonstrate the result significantly. A related finding has been that 'parameter B', the extrapolated value for  $P_{\text{ETCO}_2}$  at which ventilation is

equal to zero, fell following 8 h of hypoxia. This was not found in earlier, smaller scale studies of the response to  $\text{CO}_2$  after 8 h of hypoxia (Fatemian & Robbins, 1998, 2001), possibly because of inadequate power in these studies.

### Sex difference in pulmonary vascular acclimatization to hypoxia

A striking result of this study in relation to the pulmonary vascular response to 8 h of hypoxia was the observation that there was a difference between the sexes, with females exhibiting a greater response to the sustained hypoxia than males. It is notable that this was observed despite not limiting the study of females to one phase of the menstrual cycle. There are, of course, many factors that could underlie the sex difference in response. However, it is worthy of note that values for serum ferritin and transferrin saturation are lower in women than in men (Koziol *et al.* 2001), and that iron availability is an important factor in the rate of degradation of HIF (Wang & Semenza, 1993). In relation to this, a number of studies have now demonstrated both that iron chelation can increase PASP (Balanos *et al.* 2002) and that iron administration can substantially reduce the pulmonary vascular response to sustained hypoxia (Smith *et al.* 2008, 2009).

### Limitations in power and scope of the study

The recruitment of 46 females in a total of 80 volunteers to the study without regard to the phase of the menstrual cycle in which they were studied may have reduced the power of our study to detect correlations between measures of ventilatory and pulmonary vascular responses to hypoxia. This is because both chemoreflex responses and pulmonary vascular responses to hypoxia are known to vary through the menstrual cycle and may differ between the sexes (Dempsey *et al.* 1986; Dutton *et al.* 1989; Lahm *et al.* 2007), and variability amongst females may have been greater in this study than if they had been investigated in only one phase of the menstrual cycle. The results from the 34 males within the study were analysed separately, and no markedly different conclusions were reached compared with those from the whole data set, although, as previously noted, there was a substantial difference between the sexes in the pulmonary vascular acclimatization to hypoxia.

Finally, it should be emphasized that this study has been concerned with effects that occur early in the acclimatization response to hypoxia. Longer durations of hypoxia will elicit further effects. For example, longer durations of hypoxia may involve modulation of the carotid chemoreflex within the CNS (Dwinell & Powell, 1999), and compensatory effects in response to the initial

respiratory alkalosis may become significant (Cruz *et al.* 1980).

## References

- Albert TJ & Swenson ER (2014). Peripheral chemoreceptor responsiveness and hypoxic pulmonary vasoconstriction in humans. *High Alt Med Biol* **15**, 15–20.
- Ang SO, Chen H, Hirota K, Gordeuk VR, Jelinek J, Guan Y, Liu E, Sergueeva AI, Miasnikova GY, Mole D, Maxwell PH, Stockton DW, Semenza GL & Prchal JT (2002). Disruption of oxygen homeostasis underlies congenital Chuvash polycythemia. *Nat Genet* **32**, 614–621.
- Balanos GM, Dorrington KL & Robbins PA (2002). Desferrioxamine elevates pulmonary vascular resistance in humans: potential for involvement of HIF-1. *J Appl Physiol* (1985) **92**, 2501–2507.
- Balanos GM, Talbot NP, Robbins PA & Dorrington KL (2005). Separating the direct effect of hypoxia from the indirect effect of changes in cardiac output on the maximum pressure difference across the tricuspid valve in healthy humans. *Pflugers Arch* **450**, 372–380.
- Bärtsch P & Swenson ER (2013). Acute high-altitude illnesses. *New Engl J Med* **368**, 2294–2302.
- Beall CM, Cavalleri GL, Deng L, Elston RC, Gao Y, Knight J, Li C, Li JC, Liang Y, McCormack M, Montgomery HE, Pan H, Robbins PA, Shianna KV, Tam SC, Tsering N, Veeramah KR, Wang W, Wangdui P, Weale ME, Xu Y, Xu Z, Yang L, Zaman MJ, Zeng C, Zhang L, Zhang X, Zhaxi P & Zheng YT (2010). Natural selection on *EPAS1* (*HIF2α*) associated with low hemoglobin concentration in Tibetan highlanders. *Proc Natl Acad Sci USA* **107**, 11459–11464.
- Bisgard GE, Busch MA & Forster HV (1986). Ventilatory acclimatization to hypoxia is not dependent on cerebral hypocapnic alkalosis. *J Appl Physiol* (1985) **60**, 1011–1015.
- Brimiouille S, Vachiery JL, Brichtant JF, Delcroix M, Lejeune P & Naeije R (1997). Sympathetic modulation of hypoxic pulmonary vasoconstriction in intact dogs. *Cardiovasc Res* **34**, 384–392.
- Brooks JTS, Elvidge GP, Glenney L, Gleadle JM, Liu C, Ragoussis J, Smith TG, Talbot NP, Winchester L, Maxwell PH & Robbins PA (2009). Variations within oxygen-regulated gene expression in humans. *J Appl Physiol* (1985) **106**, 212–220.
- Busch MA, Bisgard GE & Forster HV (1985). Ventilatory acclimatization to hypoxia is not dependent on arterial hypoxemia. *J Appl Physiol* (1985) **58**, 1874–1880.
- Chapleau MW, Wilson B, Gregory TJ & Levitzky MG (1988). Chemoreceptor stimulation interferes with regional hypoxic pulmonary vasoconstriction. *Respir Physiol* **71**, 185–200.
- Clement ID & Robbins PA (1993). Dynamics of the ventilatory response to hypoxia in humans. *Respir Physiol* **92**, 253–275.
- Croft QPP, Formenti F, Talbot NP, Lunn D, Robbins PA & Dorrington KL (2013). Variations in alveolar partial pressure for carbon dioxide and oxygen have additive not synergistic acute effects on human pulmonary vasoconstriction. *PLoS One* **8**, e67886.
- Cruz JC, Reeves JT, Grover RF, Maher JT, McCullough RE, Cymerman A & Denniston JC (1980). Ventilatory acclimatization to high altitude is prevented by CO<sub>2</sub> breathing. *Respiration* **39**, 121–130.
- Daly I de B & Daly M de B (1959). The effects of stimulation of the carotid body chemoreceptors on the pulmonary vascular bed in the dog: the 'vasosensory controlled perfused living animal' preparation. *J Physiol* **148**, 201–219.
- DeGoede J, Berkenbosch A, Ward DS, Bellville JW & Olievier CN (1985). Comparison of chemoreflex gains obtained with two different methods in cats. *J Appl Physiol* (1985) **59**, 170–179.
- Dehnert C, Grunig E, Mereles D, vonLennep N & Bärtsch P (2005). Identification of individuals susceptible to high-altitude pulmonary oedema at low altitude. *Eur Respir J* **25**, 545–551.
- Dempsey JA, Olson EB & Skatrud JB (1986). Hormones and neurochemicals in the regulation of breathing. In *Handbook of Physiology, section 3, The Respiratory System*, vol. II, *Control of Breathing*, part 1, chap. 7, pp. 181–221. American Physiological Society, Bethesda, MD, USA.
- Dorrington KL, Clar C, Young JD, Jonas M, Tansley JG & Robbins PA (1997). Time course of the human pulmonary vascular response to 8 hours of isocapnic hypoxia. *Am J Physiol* **273**, H1126–H1134.
- Douglas CG, Haldane JS, Henderson Y & Schneider EC (1913). Physiological observations made on Pike's Peak, Colorado, with special reference to adaptation to low barometric pressure *Philos Trans R Soc Lond B Biol Sci* **203**, 185–318.
- Downing SE & Lee JC (1980). Nervous control of the pulmonary circulation. *Annu Rev Physiol* **42**, 199–210.
- DuBois D & DuBois EF (1916). A formula to estimate the approximate surface area if height and weight be known. *Arch Intern Med* **17**, 863–871.
- Dutton K, Blanksby BA & Morton AR (1989). CO<sub>2</sub> sensitivity changes during the menstrual cycle. *J Appl Physiol* (1985) **67**, 517–522.
- Dwinell MR & Powell FL (1999). Chronic hypoxia enhances the phrenic nerve response to arterial chemoreceptor stimulation in anesthetized rats. *J Appl Physiol* (1985) **87**, 817–823.
- Easton PA, Slykerman LJ & Anthonisen NR (1986). Ventilatory response to sustained hypoxia in normal adults. *J Appl Physiol* (1985) **61**, 906–911.
- Fatemian M, Dahan A, Meinesz S, Mey AVD & Robbins PA (2001). Modelling the ventilatory response to variations in end-tidal PCO<sub>2</sub> in patients who have undergone bilateral carotid body resection. In *Frontiers in Modeling and Control of Breathing*, ed. Poon C-S & Kazemi H, pp. 275–277. Plenum Publishers, New York.
- Fatemian M, Nieuwenhuijs DJF, Teppema LJ, Meinesz S, van der Mey AGL, Dahan A & Robbins PA (2003). The respiratory response to carbon dioxide in humans with unilateral and bilateral resections of the carotid bodies. *J Physiol* **549**, 965–973.
- Fatemian M & Robbins PA (1998). Human ventilatory response to CO<sub>2</sub> after 8 h of isocapnic or poikilocapnic hypoxia. *J Appl Physiol* (1985) **85**, 1922–1928.

- Fatemian M & Robbins PA (2001). Selected contribution: chemoreflex responses to CO<sub>2</sub> before and after an 8-h exposure to hypoxia in humans. *J Appl Physiol* (1985) **90**, 1607–1614.
- Forster HV, Bisgard GE, Rasmussen B, Orr JA, Buss DD & Manohar M (1976). Ventilatory control in peripheral chemoreceptor-denervated ponies during chronic hypoxemia. *J Appl Physiol* **41**, 878–885.
- Groves BM, Reeves JT, Sutton JR, Wagner PD, Cymerman A, Malconian MK, Rock PB, Young PM & Houston CS (1987). Operation Everest II: elevated high-altitude pulmonary resistance unresponsive to oxygen. *J Appl Physiol* (1985) **63**, 521–530.
- Hebb C (1966). Motor innervation of the pulmonary blood vessels of mammals. In *Pulmonary Circulation and Interstitial Space*, ed. Fishman AP & Hecht HH, pp. 195–222. University of Chicago Press, Chicago.
- Hohenhaus E, Paul A, McCullough RE, Kucherer H & Bärtzsch P (1995). Ventilatory and pulmonary vascular response to hypoxia and susceptibility to high altitude pulmonary oedema. *Eur Respir J* **8**, 1825–1833.
- Hoiland RL, Foster GE, Donnelly J, Stembridge M, Willie CK, Smith KJ, Lewis NC, Lucas SJE, Cotter JD, Yeoman DJ, Thomas KN, Day TA, Tymko MM, Burgess KR & Ainslie PN (2014). Chemoreceptor responsiveness at sea level does not predict the pulmonary pressure response to high altitude. *Chest* (in press; DOI: 10.1378/chest.14-1992).
- Howard LSGE, Barson RA, Howse BPA, McGill TR, McIntyre ME, O'Connor DF & Robbins PA (1995). Chamber for controlling the end-tidal gas tensions over sustained periods in humans. *J Appl Physiol* (1985) **78**, 1088–1091.
- Howard LSGE & Robbins PA (1995a). Alterations in respiratory control during 8 h of isocapnic and poikilocapnic hypoxia in humans. *J Appl Physiol* (1985) **78**, 1098–1107.
- Howard LSGE & Robbins PA (1995b). Ventilatory response to 8 h of isocapnic and poikilocapnic hypoxia in humans. *J Appl Physiol* (1985) **78**, 1092–1097.
- Kline DD, Peng YJ, Manalo DJ, Semenza GL & Prabhakar NR (2002). Defective carotid body function and impaired ventilatory responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1 $\alpha$ . *Proc Natl Acad Sci USA* **99**, 821–826.
- Kozioł JA, Ho NJ, Felitti VJ & Beutler E (2001). Reference centiles for serum ferritin and percentage of transferrin saturation, with application to mutations of the HFE gene. *Clin Chem* **47**, 1804–1810.
- Kronenberg RS, Safar P, Lee J, Wright F, Noble W, Wahrenbrock E, Hickey R, Nemoto E & Severinghaus JW (1971). Pulmonary artery pressure and alveolar gas exchange in man during acclimatization to 12,470 ft. *J Clin Invest* **50**, 827–837.
- Kummer W (2011). Pulmonary vascular innervation and its role in responses to hypoxia: size matters! *Proc Am Thorac Soc* **8**, 471–476.
- Lahiri S, Edelman NH, Cherniack NS & Fishman AP (1981). Role of carotid chemoreflex in respiratory acclimatization to hypoxaemia in goat and sheep. *Respir Physiol* **46**, 367–382.
- Lahm T, Patel KM, Crisostomo PR, Markel TA, Wang M, Herring C & Meldrum DR (2007). Endogenous estrogen attenuates pulmonary artery vasoreactivity and acute hypoxic pulmonary vasoconstriction: the effects of sex and menstrual cycle. *Am J Physiol Endocrinol Metab* **293**, E865–E871.
- Levitzky MG (1979). Chemoreceptor stimulation and hypoxic pulmonary vasoconstriction in conscious dogs. *Respir Physiol* **37**, 151–160.
- Levitzky MG, Newell JC & Dutton RE (1978). Effect of chemoreceptor denervation on the pulmonary vascular response to atelectasis. *Respir Physiol* **35**, 43–51.
- Liu C, Smith TG, Balanos GM, Brooks J, Crosby A, Herigstad M, Dorrington KL & Robbins PA (2007). Lack of involvement of the autonomic nervous system in early ventilatory and pulmonary vascular acclimatization to hypoxia in humans. *J Physiol* **579**, 215–225.
- Lodato RF, Michael JR & Murray PA (1988). Absence of neural modulation of hypoxic pulmonary vasoconstriction in conscious dogs. *J Appl Physiol* (1985) **65**, 1481–1487.
- Maggiorini M, Bärtzsch P & Swenson ER (2014). Pulmonary circulation. In *High Altitude: Human Adaptation to Hypoxia*, ed. Swenson ER & Bärtzsch P, pp. 84–102. Springer, New York.
- Maxwell PH, Pugh CW & Ratcliffe PJ (1993). Inducible operation of the erythropoietin 3' enhancer in multiple cell lines: evidence for a widespread oxygen-sensing mechanism. *Proc Natl Acad Sci USA* **90**, 2423–2427.
- Naeije R (2004). Pulmonary vascular function. In *Pulmonary Circulation: Diseases and their Treatment*, ed. Peacock AJ & Rubin LJ, pp. 3–13. Arnold, London.
- Naeije R, Lejeune P, Leeman M, Melot C & Closset J (1989). Pulmonary vascular responses to surgical chemodenervation and chemical sympathectomy in dogs. *J Appl Physiol* (1985) **66**, 42–50.
- Peacock AJ, Challenor V & Sutherland G (1990). Estimation of pulmonary artery pressure by Doppler echocardiography in normal subjects made hypoxic. *Respir Med* **84**, 335–337.
- Pedersen ME, Fatemian M & Robbins PA (1999). Identification of fast and slow ventilatory responses to carbon dioxide under hypoxic and hyperoxic conditions in humans. *J Physiol* **521**, 273–287.
- Pellett AA, Cairo JM & Levitsky MG (1997). Hypoxemia and hypoxic pulmonary vasoconstriction: autonomic nervous system versus venous P<sub>O<sub>2</sub></sub>. *Respir Physiol* **109**, 249–260.
- Powell FL, Milsom WK & Mitchell GS (1998). Time domains of the hypoxic ventilatory response. *Respir Physiol* **112**, 123–134.
- Ren X, Fatemian M & Robbins PA (2000). Changes in respiratory control in humans induced by 8 h of hyperoxia. *J Appl Physiol* (1985) **89**, 655–662.
- Robbins PA, Swanson GD & Howson MG (1982). A prediction-correction scheme for forcing alveolar gases along certain time courses. *J Appl Physiol Respir Environ Exerc Physiol* **52**, 1353–1357.
- Semenza GL & Wang GL (1992). A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. *Mol Cell Biol* **12**, 5447–5454.



- Shimoda LA, Manalo DJ, Sham JS, Semenza GL & Sylvester JT (2001). Partial HIF-1 $\alpha$  deficiency impairs pulmonary arterial myocyte electrophysiological responses to hypoxia. *Am J Physiol Lung Cell Mol Physiol* **281**, L202–L208.
- Simonson TS, Yang Y, Huff CD, Yun H, Qin G, Witherspoon DJ, Bai Z, Lorenzo FR, Xing J, Jorde LB, Prchal JT & Ge R (2010). Genetic evidence for high-altitude adaptation in Tibet. *Science* **329**, 72–75.
- Smith TG, Balanos GM, Croft QPP, Talbot NP, Dorrington KL, Ratcliffe PJ & Robbins PA (2008). The increase in pulmonary arterial pressure caused by hypoxia depends on iron status. *J Physiol* **586**, 5999–6005.
- Smith CA, Bisgard GE, Nielsen AM, Daristotle L, Kressin NA, Forster HV & Dempsey JA (1986). Carotid bodies are required for ventilatory acclimatization to chronic hypoxia. *J Appl Physiol* (1985) **60**, 1003–1010.
- Smith TG, Brooks JT, Balanos GM, Lappin TR, Layton DM, Leedham DL, Liu C, Maxwell PH, McMullin MF, McNamara CJ, Percy MJ, Pugh CW, Ratcliffe PJ, Talbot NP, Treacy M & Robbins PA (2006). Mutation of von Hippel-Lindau tumour suppressor and human cardiopulmonary physiology. *PLoS Med* **3**, e290.
- Smith TG, Talbot NP, Privat C, Rivera-Ch M, Nickol AH, Ratcliffe PJ, Dorrington KL, León-Velarde F & Robbins PA (2009). Effects of iron supplementation and depletion on hypoxic pulmonary hypertension two randomized controlled trials. *JAMA* **302**, 1444–1450.
- Swenson ER (2013). Hypoxic pulmonary vasoconstriction. *High Alt Med Biol* **14**, 101–110.
- Sylvester JT, Shimoda LA, Aaronson PI & Ward JPT (2012). Hypoxic pulmonary vasoconstriction. *Physiol Rev* **92**, 367–520.
- Talbot NP, Balanos GM, Dorrington KL & Robbins PA (2005). Two temporal components within the human pulmonary vascular response to approximately 2 h of isocapnic hypoxia. *J Appl Physiol* (1985) **98**, 1125–1139.
- Wang GL & Semenza GL (1993). Desferrioxamine induces erythropoietin gene-expression and hypoxia-inducible factor-1 DNA-binding activity – implications for models of hypoxia signal-transduction. *Blood* **82**, 3610–3615.
- Weizhen N, Engwall MJA, Daristotle L, Pizarro J & Bisgard GE (1992). Ventilatory effects of prolonged systemic (CNS) hypoxia in awake goats. *Respir Physiol* **87**, 37–48.
- Wilson LB & Levitzky MG (1989). Chemoreflex blunting of hypoxic pulmonary vasoconstriction is vagally mediated. *J Appl Physiol* (1985) **66**, 782–791.
- Yi X, Liang Y, Huerta-Sanchez E, Jin X, Cuo ZXP, Pool JE, Xu X, Jiang H, Vinckenbosch N, Korneliussen TS, Zheng HC, Liu T, He WM, Li K, Luo RB, Nie XF, Wu HL, Zhao MR, Cao HZ, Zou J, Shan Y, Li SZ, Yang Q, Asan, Ni PX, Tian G, Xu JM, Liu XA, Jiang T, Wu RH, Zhou GY, Tang MF, Qin JJ, Wang T, Feng SJ, Li GH, Huasang, Luosang JB, Wang W, Chen F, Wang YD, Zheng XG, Li Z, Bianba ZM, Yang G, Wang XP, Tang SH, Gao GY, Chen Y, Luo Z, Gusang L, Cao Z, Zhang QH, Ouyang WH, Ren XL, Liang HQ, Zheng HS, Huang YB, Li JX, Bolund L, Kristiansen K, Li YR, Zhang Y, Zhang XQ, Li RQ, Li SG, Yang HM, Nielsen R, Wang J & Wang JA (2010). Sequencing of 50 human exomes reveals adaptation to high altitude. *Science* **329**, 75–78.
- Yu AY, Shimoda LA, Iyer NV, Huso DL, Sun X, McWilliams R, Beaty T, Sham JS, Wiener CM, Sylvester JT & Semenza GL (1999). Impaired physiological responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1 $\alpha$ . *J Clin Invest* **103**, 691–696.

## Additional information

### Competing interests

No conflicts of interest, financial or otherwise, are declared by the authors.

### Author contributions

The experiments were performed in the Department of Physiology, Anatomy and Genetics at the University of Oxford. The contribution of each author to the study is as follows. Conception and design of the experiments: M. Fatemian and P.A.R. Collection, assembly, analysis and interpretation of data: M. Fatemian, M.H., Q.P.P.C., F.F., R.C., C.W., T.G.S., M. Friedmannova, K.L.D. and P.A.R. Drafting the article or revising it critically for important intellectual content: M. Fatemian, T.G.S., K.L.D. and P.A.R. All authors approved the final version of the manuscript. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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